SYNOPSIS

The role of mitochondria is multidimensional and ranges in vast areas, including apoptosis, cellular response towards stress, metabolism, which is regulated by a plethora of proteins, acting together to maintain cellular and *organellar* homeostasis. In spite of the presence of mitochondrial DNA, most of the mitochondrial proteins are nuclear encoded and translocated inside the *organelle* through dedicated translocases present on outer and inner membrane of mitochondria. To fulfill the cellular energy demand, mitochondria efficiently generate ATP by oxidative phosphorylation, and thus are considered as "*power house of cell*." There occurs a transfer of electrons from various oxidizable substrates to oxygen, which is achieved by a series of redox reactions with generation of water as a byproduct. This process is coupled with ATP synthesis, involves five protein-complexes present in the inner mitochondrial membrane. During this process, it generates extremely reactive intermediate species of oxygen as a byproduct collectively referred as Reactive Oxygen Species (ROS) through partial reduction of oxygen. These intermediate metabolites of oxygen include superoxide anion (O_2^{-o}), H_2O_2 and highly reactive hydroxyl radicals (OH^o). Although ROS are produced by different cellular sources, such as widely expressed and evolutionary conserved NADPH Oxidases, xanthine oxidase, cyclooxygenases, lipoxygenases and cytochrome P450 enzymes but mitochondria are one of the major contributors of cellular ROS.

Earlier, reactive oxygen species were considered as harmful but for past few decades, the role ROS has been appreciated as signaling molecules. Because of their high reactivity, these species can cause redox mediated modifications to cellular components and thus have an ability to participate in signaling process. The regulation of signaling pathway by ROS is governed by either alterations in cellular redox conditions or by oxidative modifications of certain residues in proteins, which are involved in signaling cascades. Reactive Oxygen Species can modify amino acid residues, interact with Fe-S clusters or other metal complexes and induce dimerization of proteins to alter protein structure and function. ROS causes modifications to critical

amino acids, mainly by oxidation of cysteine residues, where oxidation of sulfhydryl group (-SH) of a single cysteine residue leads to formation of sulfenic (-SOH), sulfinic (-SO_2H), sulfonic (-SO_3H), or S-glutathionylated (-SSG) derivatives. Thus, by incorporating these modifications, ROS affects the function of proteins, thereby modulating the cellular signaling process.

On the other hand, the accumulation of higher level of reactive oxygen species may damage cellular components causing oxidative stress. Therefore, it is necessary to maintain the ROS levels and regulation of intracellular redox homeostasis depends upon a complex network of antioxidant molecules. These antioxidants range from low molecular weight glutathione to large proteins like glutathione peroxidases. Cell has an array of antioxidants with different subcellular locations. Superoxide Dismutase which catalyzes dismutation of superoxides and converts them to H_2O_2, localizes in cytosol, mitochondrial intermembrane space and extracellular matrix. Different isoforms of Glutatione Peroxidases (GPx) and Peroxiredoxins (Prx) are located in cytosol as well as in mitochondria and scavenge H_2O_2 by using glutathione (GSH) and thioredoxin (Trx) respectively, as co-factors. During this peroxidase activity of GPx and Prx, GSH and Trx get oxidized and recycled back to the reduced form by Glutathione Reductase (GR) and Thioredoxin Reductase (TR) correspondingly, with the help of NADPH. Thus, GPx system (GPx, GR, GSH and NADPH) and Prx system (Prx, Trx, TR and NADPH) helps in maintenance of redox balance by scavenging H_2O_2. Catalase is present in peroxisomes for the catalytic degradation of H_2O_2. Along with Thioredoxin, glutaredoxin (Grx) also reduces protein disulfides and maintains the redox homeostasis.

Although, reactive oxygen species are important for normal physiological process, oxidative stress caused by imbalanced ROS levels is thought to be involved in progression of many disorders. However, in most of the diseases, the role of ROS is not yet clear. Elevated oxidative stress is observed with insulin resistance and progression of type II diabetes mellitus, and the resultant high glucose levels alter mitochondrial physiology, leading to the fragmentation of *organelle*. However, on contrary it has also been observed that ROS improves insulin sensitivity. ROS is directly involved in progression of neurodegenerative disorders, which are characterized by oxidative stress

mediated neuronal loss. Interestingly, in case of cancer ROS plays a differential role. At moderately higher levels, ROS helps cancer cells to detach from the matrix and thus assist in metastasis but the higher accumulation of ROS leads to oxidative stress mediated cell death. Thus, cancer cells have an enhanced expression level of antioxidants to maintain the optimum ROS concentration for their survival and proliferation.

The role of ROS in cellular signaling and progression of diseases highlights the importance of redox regulation. Mitochondria being the major source of ROS, harbors various redox regulators such as a mitochondrial permeability transition pore (mPTP), inner membrane anion channel (IMAC), Ca^{++} ions, etc. In addition, certain proteins like Hsp31/DJ1 class also translocates into the *organelle* in a stress dependent manner to maintain redox homeostasis. These proteins are encoded by the nuclear genome and translocated in the *organelle*, suggesting the importance of mitochondrial import machinery in regulation of redox balance. Another such example is MIA pathway of protein import, where MIA40 regulates ROS indirectly by catalyzing folding of disulfide containing proteins such as SOD-1 in a redox coupled process.

However, under most cases, the physiological disorders lead to uncontrolled production of reactive oxygen species, thereby overloading the cellular antioxidant defense machinery. The failure of the antioxidant machinery leads to enhanced disease progression. Under such disease conditions where the upheaval of redox homeostasis leads to the accumulation of ROS, artificial antioxidants can be used to protect cells against oxidative damage. Artificial systems such as Cyclodextrins, metal complexes, porphyrins, polymers, supramolecules and biomolecules such as nucleic acids, catalytic antibodies and proteins, have been created to mimic the structures and functions of natural enzymes through various approaches.

In the present thesis, we have elucidated the role of two mitochondrial proteins, which are part of mitochondrial import motor, as redox regulators and the effect of artificial antioxidants in maintenance of redox homeostasis under stress. A detailed description on importance of ROS in cellular signaling and disease progression has

been included in **Chapter I**, which gives a preface for the work mentioned in this thesis. **Chapter II to chapter V** elucidates the main objectives of the present thesis, which are:

1. Identification of novel human mitochondrial regulators of redox homeostasis

    - Role of NEF in redox sensing **(Chapter II)**
    - Evolved function of J-like protein in ROS regulation **(Chapter III)**

2. Characterization of potential artificial antioxidants as redox therapeutics

    - Organo-selenium compounds as potential artificial antioxidants **(Chapter IV)**
    - Use of nanoparticles as a natural antioxidant mimics **(Chapter V)**

Chapter II: Mitochondrial Hsp70 (mtHsp70) plays a critical role for the import of the precursor proteins. The import activity of mtHsp70 is attributed by cyclic binding and release of precursor proteins which in turn is regulated by co-chaperones J-proteins and nucleotide exchange factor (NEF). The affinity for substrate is governed by the binding of ADP or ATP at the N-terminal nucleotide binding pocket of mtHsp70. The affinity for substrate is higher in ADP bound state as compared to ATP bound state. mtHsp70 by its ATPase activity hydrolyze ATP (low-affinity state) to ADP (high-affinity state), which is replaced back to ATP by NEF thus maintaining the mtHsp70 cycle for protein import.

In the present study, we have biochemically and functionally characterized GrpEL1 and GrpEL2 as a nucleotide exchange factor for mtHsp70. We observed that like their yeast ortholog Mge1, both the mammalian NEFs interacts with mtHsp70 and exchange ADP from ATP to maintain the cycle of mtHsp70. Interestingly, we observed that both the NEFs are part of human mitochondrial import motor and are recruited at the import motor as hetero-subcomplex. The formation of GrpEL1-EL2 hetero-subcomplex is important to maintain the stability of both the NEFs. In this study, we have elucidated that the interplay between the two NEFs governs *organellar* response towards oxidative stress.

Chapter III: Redox imbalance generates multiple cellular damages leading to oxidative stress mediated pathological conditions such as neurodegenerative diseases, diabetes, ageing and cancer progression. Therefore, maintenance of ROS homeostasis is most important, that involves well-defined antioxidant machinery. In the present chapter, we have identified for first time a component of mammalian protein translocation machinery, Magmas, to perform a critical ROS regulatory function. Magmas overexpression has been reported in highly metabolically active tissues, cancer cells and tissues of developmental origin that are prone to oxidative damage. We found that Magmas regulates cellular ROS levels by controlling its production as well as scavenging. Magmas promotes cellular tolerance towards oxidative stress by enhancing antioxidant enzyme activity, thus preventing induction of apoptosis and damage to cellular components. Magmas enhances the activity of ETC-complexes, causing reduced ROS production. Our results suggest that J-like domain of Magmas is essential for maintenance of redox balance. The function of Magmas as an ROS sensor was found to be independent of its role in protein import, underlying its dual role in human mitochondria. The unique ROS modulatory role of Magmas is highlighted by its ability to increase cellular tolerance to oxidative stress even in yeast model organism. The cytoprotective capability of Magmas against oxidative damage makes it an important candidate for future investigation in therapeutics of oxidative stress related diseases.

Chapter IV: The dysregulation of antioxidant machinery in oxidative stress mediated disorders lead to accumulation of excess ROS, highlighting the importance of artificial antioxidants. For the therapeutics of oxidative stress related disorders, artificial antioxidants have been used as combination redox therapy. In order to realize potent biocompatible antioxidants with minimum toxicity, we have utilized two approaches – synthesis of organic compounds and nanoparticle based enzyme mimetics. We have synthesized novel isoselenazoles with high glutathione peroxidase (GPx) and peroxiredoxin (Prx) activities, which provide remarkable cytoprotection to human cells, mainly by exhibiting antioxidant activities in the presence of cellular thiols. The cytotoxicity of the isoselenazoles is found to be significantly lower than that of ebselen, which is being widely clinically evaluated by several research groups for the treatment

of reperfusion injuries and stroke, hearing loss, and bipolar disorder. The compounds reported in this study has the potential to be used as therapeutic agents for disorders mediated by reactive oxygen species..

Chapter V: Nanomaterials with enzyme-like properties have attracted significant interest, although limited information is available on their biological activities in cells. Here, we show that V_2O_5 nanowires (Vn) functionally mimic the antioxidant enzyme, glutathione peroxidase by using cellular glutathione as a co-factor. Although a bulk V_2O_5 is known to be toxic to the cells, the property is altered when converted into a nanomaterial form. The Vn nanozymes readily internalize into mammalian cells of multiple origins (kidney, neuronal, prostate, cervical) and exhibit robust enzyme-like activity by scavenging the reactive oxygen species, when challenged against intrinsic and extrinsic oxidative stress. The Vn nanozymes fully restore the redox balance without perturbing the cellular antioxidant defense, thus providing an important cytoprotection for biomolecules against harmful oxidative damage. Based on our findings, we envision that biocompatible Vn nanowires can provide future therapeutic potential to prevent ageing, cardiac disorders and several neurological conditions, including Parkinson's and Alzheimer's disease.